mix, and dilute with distilled water to obtain a concentration of 2,000 units per milliliter. Dilute the other portion, which is to be used as the blank solution, with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a concentration of approximately 2,000 units per milliliter.

(ii) *Penicillin G procaine content*—(*a*) *Reagents*—(*1*) *Sodium nitrite solution.* Dissolve 0.1 gram of sodium nitrite in 100 milliliters of distilled water. Prepare a fresh solution every week and store under refrigeration.

(*2*) *Ammonium sulfamate solution.* Dissolve 0.5 gram of ammonium sulfamate in 100 milliliters of distilled water and store under refrigeration.

(*3*) *N-(1-naphthyl)-ethylenediamine solution.* Dissolve 0.1 gram of *N*-(1-naphthyl) - ethylenediamine dihydrochloride in 100 milliliters of distilled water. Prepare fresh solutions every week and store under refrigeration.

(4) Standard procaine solution. Prepare a standard solution containing 27.55 milligrams of procaine hydrochloride U.S.P. in a liter of distilled water (each milliliter of the standard solution is equivalent to 60 units of penicillin G procaine).

(*b*) *Preparation of sample solution.* Using a suitable hypodermic needle and syringe, withdraw a one-dose portion of the sample and place it into an appropriate-sized volumetric flask. Add 20 milliliters of 0.5*N* NaOH for each 300,000 units of penicillin G benzathine, mix well, being sure that all penicillin is in solution, and allow to stand for 15 minutes. Add 1 milliliter of 1.2*N* HC1 for each 2 milliliters of 0.5*N* NaOH, mix, and dilute with distilled water to obtain a concentration of 60 units of penicillin G procaine per milliliter. Transfer a 3.0-milliliter aliquot of this solution to a 50-milliliter volumetric flask and add 2 milliliters of water to give a volume of 5 milliliters.

(*c*) *Procedure.* Transfer respectively, 1.0, 2.0, 3.0, 4.0, and 5.0 milliliters of the standard procaine solution to each of five 50-milliliter volumetric flasks and transfer 5.0 milliliters of distilled water to a sixth 50-milliliter volumetric flask. Add 4.0, 3.0, 2.0, and 1.0 milliliters of water to the first four flasks, respectively, to give each a volume of 5 milliliters. To each flask of the standard and sample solutions, add 0.5 milliliter of 4*N* HC1, 1.0 milliliter of sodium nitrite solution, 1.0 milliliter of ammonium sulfamate solution, and 1.0 milliliter of *N*-(1-naphthyl)-ethylenediamine solution. Mix and wait two minutes after each addition. Dilute each flask to volume with distilled water. Using a suitable photoelectric colorimeter, determine the absorbancy of each solution at 550 nanometers. The instrument is balanced so that the zero concentration reads 0 absorbancy. Plot the standard curve on coordinate graph paper. Obtain the procaine penicillin content of the solution for assay directly from the point on the standard curve corresponding to its absorbancy.

(iii) *Penicillin G benzathine content.* The sum of the penicillin G procaine content determined as directed in paragraph (b)(1)(ii) of this section subtracted from the total potency determined as directed in paragraph (b)(1)(i) of this section represents the penicillin G benzathine content.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, medium F in lieu of medium E, and during the period of incubation, shake the tubes at least once daily.

(3) *Pyrogens.* Proceed as directed in §436.32(d) of this chapter, using a solution containing 4,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted aqueous suspension.

[42 FR 59868, Nov. 22, 1977, as amended at 43 FR 9799, Mar. 10, 1978; 50 FR 19918, 19919, May 13, 1985]

§440.255d Sterile penicillin G benzathine for suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile penicillin G benzathine for suspension is a dry mixture of penicillin G benzathine and one or more suitable suspending or dispersing agents, and with or without one or more suitable preservatives and buffer substances. Its potency is satisfactory if it is not less than 90 percent and not

more than 115 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not less than 5.0 percent and not more than 8.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The penicillin G benzathine used conforms to the standards prescribed by § 440.55a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G benzathine used in making the batch for potency, moisture, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, moisture, and pH.

(ii) Samples required:

(*a*) The penicillin G benzathine used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) If the batch is packaged for repacking:

(*i*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*ii*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(*2*) If the batch is packaged for dispensing:

(*i*) For all tests except sterility: A minimum of 10 immediate containers.

(*ii*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single–dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dissolve the portion thus obtained with sufficient absolute methyl alcohol to give a solution of convenient concentration. Immediately, further dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Using the sample thus obtained, proceed as directed in § 436.205(b)(2) of this chapter.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E. During the period of incubation shake the tubes at least once daily.

(3) *Pyrogens.* Proceed as directed in § 436.32(d) of this chapter, using a solution containing 4,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using the suspension obtained when the product is reconstituted as directed in the labeling.

[42 FR 59869, Nov. 22, 1977, as amended at 50 FR 19918, 19919, May 13, 1985]

§ 440.274 Penicillin G procaine injectable dosage forms.

§ 440.274a Sterile penicillin G procaine with aluminum stearate suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*